B-131.

...herence of *Vibrio cholerae* to Differentiated Human Intestinal Cells in Vitro

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...vious studies on adherence of *Vibrio cholerae* using animal models, ...ured cell lines, human tissues and volunteers have revealed a large ...mber of factors which may contribute to colonization including various ...magglutinins, pili, outer membrane proteins and LPS. In the systems used ...dence suggests the involvement or essentiality, but not necessarily the ...ficiency, of each in the adherence/colonization process. Use of animal ...ues, fixed tissue, and undifferentiated cell lines may not faithfully reflect ...e interactions between cholera vibrios and the highly differentiated cells ...ch line the human small bowel. In the present study we examine adher- ...ce of *V. cholerae* to highly differentiated columnar monolayers of the ...cus-secreting goblet cell line HT29-18N2. HT29-18N2 cells, grown on 13 ...m diameter cover slips, were induced to differentiate by switching to ...-free hybridoma medium for 10-14 days, inoculated with *V. cholerae* ... El Tor/classical biotypes and mutants, as well as serogroup O139, and ...ubated for varying times. The cover slips were washed to remove unbound ...teria and exposed to 1% Triton X-100 to lyse the HT29-18N2 cells and ...erate the attached vibrios which were enumerated by dilution plating. ...roscopic observations indicated that the vibrios bound in a nonrandom ...t spot" fashion suggestive of tissue surface irregularities or a ...cruitment" mechanism. Although results between experiments were ...metimes variable, examination of genetically modified vibrios showed that ...ther motility, flagella, nor the "virulence cassette" were required for initial ...ding. However, mutations in the hemagglutinin/protease gene (*hap*) ...coding a putative "detachase" significantly increased binding, whereas ...R vibrios and El Tor strains lacking the mannose-sensitive hemagglutinin ...owed reduced binding in preliminary tests. Bound bacteria multiplied ...neration time ≅ 35 minutes). We propose that HT29-18N2 cells may be ...useful *in vitro* system for resolution of factors involved in *V. cholerae* ...herence and colonization.

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...herence of the Amazonia Variant of *Vibrio cholerae* 01 to Confluent ...nolayers of Cultured Human Intestinal Epithelial Caco-2 Cells.

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...survey of pathogenic *V.cholerae* 01 strains from Brazilian Amazon by ...ng AP-PCR fingerprinting revealed a non-toxigenic variant (Amazonia ...nt) distinct from the current El Tor epidemic strains (Coelho *et al.*, ...Clin.Microbiol.,33:114;1995) and negative for *ctx* , *sto*, *zot* and *tcpA* genes ...t positive for the *toxR* regulatory gene.Pathogenic strains of *V.cholerae* 01 ...aborate adhesins and outer membrane proteins (OMP) required for bacterial ...lonization of the human intestine.We developed a quantitative Caco-2 cell ...herence assay and compared adherence of Amazonia variant and El Tor ...ains isolated from the same geographical area. All strains were grown in ...ulture conditions favourable to expression of mannose-sensitive (MS) pili and ...CP and were tested in presence and absence of D-mannose and L-fucose. ...mose-stained cell monolayers did not show bacterial adherence patterns as ...ously described for other enteropathogens. Quantitative tests showed that both ...l Tor and Amazonia strains adhered avidly to cells. Depending on culture ...nditions, either Amazonia or El Tor strains performed adherence better. ...ree of 5 Amazonia strains tested had their adherence indexes decreased by ...% to 64% after addition of D-mannose. Electron microscopy of Amazonia ...ains did not reveal surface appendages identified as pili. The Amazonia ...ant also express a major OMP distinct from OmpU in molecular weight. Its ...e in adherence to Caco-2 cells is currently under investigation.

B-133. Motility of *Vibrio anguillarum* Enhances the Invasion of a Fish Cell Line

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...A fish pathogen, *Vibrio anguillarum*, has been shown to require motility for crossing ...the fish integument, one of the first steps in the onset of vibriosis in marine fish. To ...test the requirement of motility for the entry into the fish host, invasion and adher- ...ence assays were done by double immunofluorescence microscopy using Chinook ...salmon embryo-214 fish cells (CHSE-214) and a set of previously described isogenic ...flagellar mutants. For the invasion assays, total, intracellular, and extracellular bac- ...teria were counted. The wild type and the *flaC*, *flaD*, or *flaE* gene mutants with slight ...to no defects in motility showed 28% intracellular bacteria; whereas, the *flaA* mutant ...with 50% of the wild-type motility, a *motY* mutant, that has a paralyzed flagellum, ...and a mutant that lacks the flagellum totally showed 13%, 10%, and 19% intracellu- ...lar bacteria, respectively. When the *motY* and the *flaA* mutant were complemented, ...the percent of intracellular bacteria returned to that of the wild-type, 33% and 29% ...respectively. A *flaB* deletion, which has a polar effect on a downstream flagellar gene ...and which leads to an elongated flagellum and only a slight decrease in motility, ...CHSE-214 cells that had bound bacteria after extensive washing. Wild type and all of ...the *fla* mutations showed an average of 6% adherence. However, the *motY* mutant ...and the

B-134. Adherence and Internalization of *Vibrio vulnificus* and Other *Vibrio spp.* by Oyster Primary Cell Cultures

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Vibrio vulnificus (*Vv*) and other vibrios such as *V. fluvialis* (*Vf*) and *V. hollisae* (*Vh*), are Gram-negative bacterial pathogens which cause disease, such as gastroenteritis, wound infections, and septicemia in humans. Illness commonly occurs after ingestion of contaminated raw seafood, for example oysters, and by exposure of wound surfaces to seawater carrying these organisms. Unlike other enterics, *Vv* strains are recalcitrant to cleansing procedures, such as depuration. Previously, we described a fibrillae expressed by *Vv* and demonstrated its role in adherence to primary cultures (PCs) of oyster mantle, heart, and hemocyte cells. In this study, we further characterized the interactions of a wild type *Vv* strain (WT), an afibrillated mutant (Fib$^-$), as well as, *Vf* and *Vh* with oyster mantle and intestinal PCs. Adherence of a Fib$^-$ mutant to mantle and intestinal PCs was significantly less than that of the WT strain ($p < 0.05$). Both *Vf* and *Vh* adhered to each oyster PC type but at a reduced level compared to the *Vv* strain ($p < 0.05$). In an invasion assay, both *Vv* and *Vf* were internalized by mantle and intestinal cells, while the *Vh* strain was only internalized by mantle cells when compared to an *E. coli* HB101 control strain ($p < 0.05$). These results confirm that the fibrillae expressed by *Vv*, described previously, are involved in adherence to oyster primary cells. These data also demonstrate tissue preference and internalization differences by oyster PCs among *Vibrio* species.

B-135. *Perkinsus marinus* serine protease prolongs survival of *Vibrio vulnificus* in Eastern oyster hemocytes *in vitro*

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Perkinsus marinus (*Pm*), a protozoan, is responsible for mortality of Eastern oysters. It produces a serine protease that is reported to inhibit oyster hemocyte killing of intracellular *Pm* organisms in a dose-dependent fashion. *V. vulnificus* (*Vv*) is a bacterium which causes gastroenteritis and primary septicemia in humans; infection is primarily acquired by ingestion of raw oysters. Most, if not all oysters infected with *Pm* also contain *Vv*. We examined the effects of *Pm* protease on intracellular survival of *Vv* in oyster hemocytes. Freshly harvested hemocytes from *Pm*-free oysters were treated (TX) with protease 1 h prior to infection. Untreated (UNTX) and TX hemocytes were infected with *Vv* in a gentamicin internalization assay for 0, 30, 60, and 120 min. at 25°C. Results showed that at 0 min. more *Vv* were recovered from UNTX hemocytes than from TX hemocytes ($p < 0.05$). However, by 30 min. and thereafter, greater numbers of *Vv* were recovered from TX hemocytes than from UNTX hemocytes ($p < 0.05$). These results suggest that TX hemocytes were initially slower to internalize *Vv* than UNTX hemocytes, but once internalized, *Vv* bactericidal activity of TX hemocytes was suppressed. These results demonstrate that *Pm* protease may play a role as an immunomodulator of oyster hemocyte bactericidal activity, and this could explain *Vv* persistence in oysters.

B-136. Internalization of *Vibrio vulnificus* and other *Vibrio spp.* in fish primary and tissue culture cells.

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Members of the genus *Vibrio* cause disease in a variety of seafood species, as well as in humans. However, little is known about the pathogenic mechanisms involved in fish diseases. Many of the descriptions of vibriosis in fish suggest invasive, systemic disease. We examined the invasiness of *V. vulnificus* (*Vv*) and *V. fluvialis* (*Vf*) in Mummichog (*Fundulus heteroclitus*) anterior kidney and liver primary culture cells, and *Vv*, *Vf*, *V. hollisae* (*Vh*), *V. mimicus* (*Vm*), and *V. parahaemolyticus* (*Vp*) in an Atlantic Menhaden liver tissue cell line. Inhibitors of actin, microtubule, and receptor-mediated endocytosis were used to determine invasion mechanisms utilized in the liver cell line. Invasion studies demonstrated that both *Vf* and *Vv* were internalized into the Mummichog kidney and liver primary cells within 30 min. compared to an *E. coli* HB101 control strain ($p < 0.05$). Using the Menhaden liver cell line, all *Vibrio* spp., except for *Vh*, were internalized within 1 h post infection. Inhibitor studies showed that internalization of *Vm* depended on all three uptake pathways. *Vp* was internalized via both the microfilament and microtubule pathways, and internalization of *Vv* and *Vf* was dependent on only the microtubule pathway. These results demonstrate that *Vibrio* spp. invade both primary and cultured fish cells, and that internalization requirements vary among *Vibrio* spp.